APPENDIX A

THE INSTITUTE OF ENVIRONMENT AND HUMAN HEALTH OF TEXAS TECH UNIVERSITY

PERCHLORATE ANALYSIS STANDARD OPERATING PROCEDURES

The Institute of Environmental and Human Health (TIEHH)
Texas Tech University
Texas Tech University Health Sciences Center
Box 41163, Lubbock, TX 79409-1163
qa@tiehh.ttu.edu

Determination of Perchlorate in Water Using Ion Chromatography with Suppressed Conductivity Detection

Author(s):		
	Rashila Patel	Date
Facility Representative:	Todd Anderson	Date
QA Review:	Brian Birdwell	Date
Asst. Director for Science:	Lou Chiodo	Date
Director:	Ronald J. Kendall	Date

1.0 OBJECTIVE

1.1 To analyze perchlorate in reagent water, surface water, ground water, and finished drinking water using ion chromatography with suppressed conductivity detection.

1.2 The Groundwater Analysis Laboratory (GAL) has established a reagent water Method Detection Limit (MDL, referred to in section 7.1.5) for the above mentioned analyte as listed in Appendix V, Tables VII and VIII. Based on this MDL study, the Gal will report data at the Minimum Reporting Limit for perchlorate in all water samples at 4 ppb, a value slightly higher than the calculated Minimum Reporting Limit (MRL). This was done to avoid repeated QC failures at the ICCS (Initial Calibration Check Standard).

1.3 The GAL will report any perchlorate detected between the Laboratory established MDL and the Minimum Reporting Limit as “trace present” and will not be reported as a quantitated concentration.

1.4 The MDL for a different matrix may differ from the one listed above depending upon the nature of the matrix and the procedure used.

1.5 See a list of definitions for all the acronyms found in this SOP in Section 12.0.

2.0 HEALTH AND SAFETY

Proper laboratory attire including a lab coat, gloves, and safety glasses must be worn for the duration of the time spent in sample/chemical preparatory stages of the analysis. The toxicity and carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable.

Sodium Hydroxide (NaOH), used in the preparation of the chromatographic eluent, is considered caustic.

In addition to proper attire, the manufacturer's safety labels should be strictly adhered to for the handling of Sodium Chloride, Sodium Sulfate, Sodium Carbonate, Potassium Chloride, and Sodium Perchlorate powder and all other chemicals in the laboratory. Material Safety Data Sheets must be read and understood by all individuals working with any kind of chemicals.

3.0 PERSONNEL/TRAINING RESPONSIBILITIES

Any TIEHH staff member trained in the use of ion chromatography and in the interpretation of the resulting ion chromatograms may perform this procedure. Users of the method data should identify data quality objectives as specified by the sponsor prior to analysis. Users of this method must demonstrate the ability to generate acceptable results, using the procedures described herein.

4.0 REQUIRED AND RECOMMENDED MATERIALS

4.1 Equipment and Supplies

4.1.1 Ion Chromatograph (IC) – Analytical system consisting of plastic eluent reservoir bottles, a suitable liquid pump, injection valve, guard column, analytical separation column, electrical suppressor, conductivity detector, and computer based data acquisition system.

4.1.1.1 Anion Guard Column – Dionex AG16, 4 mm i.d. (P/N 55377) or equivalent.

4.1.1.2 Anion Separation Column – Dionex AS16, 4 mm i.d. (P/N 55376) or equivalent.

4.1.1.3 Anion Suppressor Device – Dionex Anion Self Regenerating Suppressor (4 mm ASRS, ULTRA, P/N 53946), or equivalent.

4.1.1.4 Detector – Conductivity Cell with temperature control (Dionex CD20 (P/N 44095), conductivity cell with temperature control (P/N 44130) or equivalent)

4.1.1.5 Chromatography Oven – Thermostat temperature control of chromatography temperature (Dionex LC25).

4.1.2 Data Acquisition System – Dionex PeakNet[6] Data Chromatography Software or equivalent.

4.1.3 Conductivity Meter - At a minimum, this meter should be capable of measuring matrix conductance over a range of 1 – 10,000 µS/cm.

4.1.4 Bottles – High density polyethylene (HDPE) or glass, amber or clear, 30 ml, 125ml, and 250 ml.

4.1.5 Particulate Filters – 0.45 micron syringe filters for IC applications (Gelman IC Acrodisc, P/N 4485, or equivalent).

4.1.6 Matrix pretreatment cartridges in the barium form – Dionex OnGuard-BA cartridges, P/N 046072, or equivalent. These cartridges are used to reduce the matrix levels of sulfate.

4.1.7 Matrix pretreatment cartridges in the silver form – Dionex OnGuard-Ag cartridges, P/N 039673, or equivalent. These cartridges are used to to reduce the matrix levels of chloride.

4.1.8 Matrix pretreatment cartridges in the hydrogen form – Dionex OnGuard-H cartridges, P/N 039596, or equivalent. These cartridges are used to reduce cations like silver (Ag) in the sample matrix that has leached from the Ag cartridge and indirectly minimizes the effect of carbonate by removing the cationic counter ion.

4.1.9 Disposable 5 mL syringes, used during sample preparation.

4.1.10 Analytical balance, accurate to +/- 0.0001g.

4.1.11 Weigh boats, spatulas in various sizes, micro-pipettes in various sizes TD (to deliver), micro-pipette tips in varying sizes, and class A volumetric flasks in varying sizes.

4.1.12 Self adhesive labels for labeling prepared standards and/or dilutions.

4.1.13 Indelible black ink pens.

NOTE* This laboratory will use the Dionex AG16 guard column with the Dionex AS16 separator column based on the recommendation of the EPA Method 314.0, section 6.1.2.1. Column comparison studies are also found in the same method in Table 4, on page 39.

4.2 **Reagents and Standards**

4.2.1 Reagent Water – Distilled, deionized water having a resistance of 18.0 Mohm or better, free from anions of interest. Water should contain particles no larger than 0.45 microns.

4.2.2 Eluent solution – 100 mM sodium hydroxide (made by dissolving 8.0 grams of 50% (W/W) sodium hydroxide in reagent water to a final volume of 1.0 L, Fisher, SS254-500).

4.2.3 Perchlorate stock solution, 100 µg/ml – A stock standard solution purchased as a certified solution from Accustandard (IS-6352-0.5X).

4.2.4 Sources of anions which are used to prepare a solution for the maximum conductivity threshold :

4.2.4.1 Sodium Chloride from Fisher Scientific, BP358-1

4.2.4.2 Sodium Sulfate, Fisher, BP354-500

4.2.4.3 Sodium Carbonate, Fisher, BP357-1

Use each of the above at 25 mg/ml anion concentrations. This solution is used to prepare simulated common anion samples in the determination of the matrix conductivity threshold (MCT).

4.2.5 Conductivity meter calibration solution – Potassium Chloride (KCl), Fisher, BP366-500

4.2.6 Sodium Perchlorate , Aldrich Chemicals, 7601-89-0 (100g). This solid powder is used to prepare a quality control standard (QCS) as a second source of perchlorate to verify the calibration.

4.3 **SOPs and Forms:**

SOPs GW-5-05, GW-4-02, GW-1-03.
Form No. 267, Daily Run Log For Perchlorates.
Form No. 296 , Sample Preparation Log.
Form Nos. 80, 81, 88, 298, and 120.

5.0 SUMMARY OF METHOD

A 1.0 ml volume of sample is introduced into an ion chromatograph (IC). Perchlorate is separated and measured, using a system comprised of an ion chromatographic pump, sample injection valve, guard column, analytical column, suppressor device, and conductivity detector.

NOTE*

This large sample loop (1.0 ml) can be made using approximately 219 cm (86 inches) of 0.03 inch i.d. PEEK tubing. The exact volume of the sample loop is not critical since all standards and samples will use the same loop. However, the volume should be verified to be within 5% of this volume by weighing the sample loop empty, filling the loop with deionized water, and reweighing the loop. Assuming the density of water to be 1.0 mg/µL can approximate the volume.

6.0 GUIDELINES

6.1 Interferences

6.1.1 Method interferences may be caused by contaminants in reagent water, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baselines in an ion chromatogram. These interferences can lead to false positive results for the target analyte as well as reduced detection limits because of elevated baseline noise.

6.1.2 Interferences can be divided into three different categories: direct chromatographic coelution, where an analyte response is observed at very nearly the same retention time as the target anion; concentration dependent coelution, which is observed when the response of higher than typical concentrations of the neighboring peak overlap into the retention window of the target anion; and ionic character displacement, where retention times may significantly shift due to the influence of high ionic strength matrices (high mineral content or hardness) overloading the exchange sites in the column and significantly shortening analyte's retention times.

6.1.2.1 Sample dilution may resolve some of the difficulties if the interference is the result of either concentration dependent coelution or ionic character displacement. Sample dilution will alter the Minimum Reporting Limit (MRL) by a proportion equivalent to that of the dilution. Therefore, careful consideration of project objectives must be given prior to performing such a dilution.

6.1.2.2 Pretreatment cartridges can be used to eliminate certain types of matrix interferences. If using any sample pretreatment, the analyst must verify that the target analyte is not affected by monitoring recovery after pretreatment and that no background contaminants are introduced during

the pretreatment process. Calibration standard chromatograms should be compared to the column test chromatogram or use the calibration chromatograms generated when the column was initially installed, to insure proper separation and similar response ratios between the target analytes are observed.

6.1.3 Sample matrices with high concentrations of common ions such as chloride, sulfate, and carbonate can cause the baseline to become destabilized in the retention time window for Perchlorate. When the Matrix Conductivity Threshold (MCT) is exceeded, procedures incorporating sample dilution and/or pretreatment must be performed as specified in the procedure section of this SOP.

6.1.4 All reagent solutions (eluents, external water for ASRS suppressor, etc.) used by the IC instrument must be filtered through no larger than a 0.45 micron nominal pore size membrane or frit to remove particulates and prevent damage to the instrument, columns, and flow systems. Sample filtration must be employed on every sample prior to analysis. This applies not only to field samples but also to the laboratory reagent blank (LRB) and laboratory fortified blank (LFB). The LRB and LFB samples function as controls and must be filtered to confirm no bias is attributable to the filtration. Filters specifically designed for IC applications should be used.

6.1.5 Close attention should be given to the potential for carry over peaks from one analysis, which will affect the proper detection of Perchlorate in the second, subsequent analysis. It is the responsibility of the analyst to confirm that no late eluting peaks have carried over into a subsequent analysis thereby compromising the integrity of the analytical results.

6.1.6 Samples containing Perchlorate need no preservative and do not need to be shipped on ice. The sample holding time for Perchlorate shall not exceed 28 days (See EPA Method 314.0, section 8.3).

7.0 PROCEDURES

7.1 Calibration and Standardization

7.1.1 Demonstration and documentation of acceptable initial calibration is required prior to the Initial Demonstration of Capability (IDC) and before any samples are analyzed, and is required intermittently throughout sample analysis to meet the required QC performance criteria outlined in this method. Initial calibration verification is performed using a Quality Control Sample (QCS) as well as with each analysis batch using an initial, continuing (when more than 10 field samples are analyzed), and end calibration check standards.

7.1.2 **Initial Calibration Curve**

7.1.2.1 Establish ion chromatographic operating parameters equivalent to those indicated in Table I (See Appendix I).

7.1.2.2 Estimate the Linear Calibration Range (LCR). The LCR should cover the expected concentration range of field samples and should not extend over two orders of magnitude in concentration. If quantitation over a larger range is necessary, two separate calibration curves should be prepared. A minimum of three calibration standards are required for a curve that extends over a single order of magnitude and a minimum of five calibration standards are required if the curve covers two orders of magnitude. The GAL will use five calibration standards in the range of 4.0 µg/L to 200 µg/L.

7.1.2.3 Prepare a blank and working calibration standards by carefully adding accurately measured volumes of the stock standard to a volumetric flask and diluting to volume with reagent water. Record all dilutions and standard preparations in appropriate logs (See SOP GW-5-05, Standards/Reagents Traceability). The lowest calibration standard must be at the laboratory reporting limit. Prepare the calibration concentrations as follows:

- Label five, 250 ml volumetric flasks with the different concentrations of the calibration standard i.e. 4.0 ppb, 50 ppb, 100 ppb, 150 ppb, 200 ppb.

- Take aliquots of the Perchlorate stock solution at 100 ppm (µg/ml) and add these to the labeled volumetric flasks. Fill each flask to volume with miliQ water according to Table II (See Appendix II).

- Pour 15 mL of a calibration standard/sample into a micro beaker. Using a 5 mL Luer lock syringe, draw up approximately 5 mL of standard, attach a 0.45 micron filter to the syringe and filter the standard, directly into the auto-sampler vial. Repeat with new filters and syringes for each sample or standard.

- Place auto-sampler vials in the correct order of ascension (starting with a blank, 4.0 ppb, followed by 50 ppb and ending with 200 ppb) in the auto-sampler tray.

- Place auto-sampler tray in the auto-sampler.

- Prepare a sequence table for the calibration standards specifying the Perchlorate method on the PeakNet[6] Software.

- Analyze the standards according to SOP GW-4-02 (General Operation

of the Dionex IC25 Ion Chromatograph). Particular attention must be given to section 5.1 of SOP GW-4-02 since degassing the eluent is a critical and necessary step for the analysis.

7.1.3 Initial Calibration Check Standard (ICCS):

Analyze your lowest calibration standard again and use it as the Initial Calibration Check Standard (ICCS) to verify your initial calibration. Percent recovery of perchlorate must be in the range of 90-110%. If the 10 % recovery criteria is not met, re-evaluate the method/system and recalibrate followed by another ICCS.

7.1.4 Verify the accuracy and the acceptability of the instrument performance by analyzing a Quality Control Sample (QCS), an externally prepared second source at a concentration level that is near the middle of the calibration curve. The standard should fall within the ± 10 % recovery rule in 7.1.3. If the check does not meet acceptance criteria, re-evaluate the method/system and recalibrate followed by a verification.

7.1.5 Establish a method detection limit (MDL) by analyzing seven replicates of laboratory fortified reagent water and process through the entire analytical method over a three day period. Follow SOP GW –1-03 for calculating the MDL.

7.1.5.1 Add 100 µL of 100 ppm stock perchlorate standard to a 25 mL volumetric flask and fill to volume to make a solution at the concentration of 400 µg/L (ppb). Take 2.5 mL of the 400 ppb intermediary standard and add it to a 25 mL volumetric flask and fill to volume to get a concentration of 2.5 ppb.

7.1.5.2 Take seven aliquots of the above dilution to make seven replicates.

7.1.5.3 Analyze the seven replicates and also record the retention time of each run to check for a reliable retention time for perchlorate.

7.1.5.4 Calculate the MDL based on the results of the above data.

7.1.5.5 Verify the MDL by taking an analyte concentration that is 2-3 times the MDL and analyzing it.

NOTE* The MDLs should be verified periodically depending on changing conditions of the instrument. Every new analyst must determine a new MDL and may not use the existing MDL determined by the previous analyst. The suggested concentrations in 7.1.5.1 may also change according to the changing conditions of the instrument.

7.1.6 Minimum Reporting Limit (MRL):

The Minimum reporting Limit should be set at 3-5 times the calculated MDL. The MRL can be set at the lowest level of the calibration curve or slightly above that. The MRL should never be set below the lowest calibration standard if an accurately quantified result is wanted. Any perchlorate recovered between the MDL and the MRL must be reported as "trace present" and flagged with the letter J as an estimated concentration.

7.1.7 **Initial Demonstration of Accuracy (IDA):**

7.1.7.1 Validate the accuracy of the method by analyzing seven replicates of laboratory fortified blanks (LFBs) fortified at 25 ppb of Perchlorate.

7.1.7.1.1 Take 25 µL of the 100 ppm stock perchlorate standard and add it a 100 mL volumetric flask. Fill to volume with reagent water to make a 25 ppb concentration.

7.1.7.2 Calculate their mean measured concentrations ($C_{\bar{x}}$) of the replicate values as follows:

$$C_{\bar{x}} = \frac{(C1 + C_2 + C_3 + \ldots C_n)}{n}$$

where,

$C_{\bar{x}}$ = Mean recovered concentration of the replicate analysis.
$C_1, C_2, \ldots C_n$ = Recovered concentrations of the replicate 1, 2, ….n.
n = 7
To pass the IDA, the value derived for $C_{\bar{x}}$ must be within 10 % of the true value or between 22.5 µg/L and 27.5 µg/L.

7.1.8 **Initial Demonstration of Precision (IDP):**

Using the data above, calculate the percent relative standard deviation (%RSD) of the replicate analysis using the formula below:

$$\% \text{ RSD} = \frac{S_{n-1}}{C_{\bar{x}}} \text{ x } 100\%$$

Where, S_{n-1} = sample standard deviation (n-1) of the replicate analyses.
$C_{\bar{x}}$ = mean recovered concentration of the replicate analysis.

The % RSD of the replicate analyses must be less than 10 %.

7.1.9 **Matrix Conductivity Threshold (MCT)**

Determine the MCT by preparing a series of sequentially increasing, common

anion fortified, reagent water samples, each containing a constant concentration of Perchlorate. Calibrate the Conductivity Meter before determining the MCT by following the procedure:

7.1.9.1 **Conductivity Meter Calibration:**

- Dissolve 0.745 g of Potassium Chloride (KCl) in reagent water and dilute to a final volume of 1.00 L in a volumetric flask (The reference conductance of this solution is 1410 µS/cm).
- Rinse the electrode in reagent water, place it in reagent water, turn on the meter and confirm that the conductance of this blank is < 1 µS/cm.
- Pour 15 mL of KCL solution into a beaker, place the electrode in the solution and measure the conductivity. (It should read between 1380 µS/cm and 1440 µS/cm to be in calibration).
- If the conductivity meter fails the calibration, recalibrate the unit per manufacture's instruction and then repeat the procedure.

7.1.9.2 Prepare a laboratory fortified reagent blank containing Perchlorate at a concentration of 25 ppb by adding 12.5 µL of the 100 ppm stock solution to a 50 mL volumetric flask and filling to volume with reagent water.

7.1.9.3 Prepare sequentially increasing ionic solutions each containing Perchlorate at the concentration of 25 ppb as follows:

7.1.9.4 Dissolve the following salts in reagent water to a final volume of 25.0 mL:

- 1.0 g sodium chloride = 0.61 g Cl^-
- 0.93 g sodium sulfate =0.63 g $SO_4^=$
- 1.1 g sodium carbonate = 0.62 g $CO_3^=$

7.1.9.5 Prepare the series of common anion fortified reagent water in 50 mL volumetric flasks as in Table III (see Appendix II)

7.1.9.6 Measure and record conductance of each of the prepared solutions on a calibrated conductivity meter (section 7.1.9.1). Use the 400 mg/L mixed anion sample as a relative reference conductance. The conductance should be between 3200 µS/cm and 3700 µS/cm.

7.1.9.7 Analyze each solution on the PeakNet Software, recording the peak area to height (A/H) ratio and the quantified concentration of Perchlorate (See Table VIX, Appendix IV).

7.1.9.8 Calculate the A/H ratio percent difference ($PD_{A/H}$) between the average A/H ratio for the LFB (A/H_{LFB}) and the average A/H ratios for each mixed common anion solutions (A/H_{MA}) using the

following equation:

$$PD_{A/H} = \frac{|(A/H_{LFB} - A/H_{MA})|}{A/H_{LFB}} \times 100$$

NOTE* As the conductivity of the matrices increases, the $PD_{A/H}$ will increase. MCT is the matrix conductance where the $PD_{A/H}$ exceeds 20%.

7.1.9.9 Perform a linear regression on these data by plotting $PD_{A/H}$ (as the independent variable, x) verses the matrix conductance (as the dependent variable, y). The regression data must yield an r^2 value of > 0.95. Record the constant (intercept value) and the " X – coefficient " (slope) and calculate the MCT as follows:

$$MCT = (20\%) \times (X\text{-coefficient}) + \text{constant}$$

7.1.9.10 Confirm the Perchlorate minimum reporting limit (MRL) by analyzing a mixed common anion solution that reflects a conductance near the above-specified MCT. The concentration of the Perchlorate must be at the laboratory determined MRL, while the concentration of the mixed common anion solution must be estimated near the MCT.

NOTE* The resulting conductance must be within ± 10% of the MCT and the measured Perchlorate must be between 80 – 120 % of the MRL concentration.

7.2 SAMPLE PREPARATION

During the preparation of all standards, special attention must be given to the expiration date of the stock perchlorate standards whether they are made in the laboratory or bought as certified standards from a manufacturer. Although the perchlorate stock standard may be stable for longer periods of time, stored at room temperature, do not store longer than 12 months. All the standards, stock and/or intermediary or working, must be labeled with an expiration date. Intermediary and working standards must be re-made after one month.

7.2.1 Bring all samples and standards to room temperature prior to analysis (in the temperature range of 24°C ± 5°C).

7.2.2 All sample batches must be accompanied by the QCS, IPCS, LRB, ICCS, LFB, LFM, LFMD, CCCS, and ECCS (See section 12.0 for definitions).

7.2.2.1 Prepare the above standards as follows:

- QCS - Weigh 0.1231 g of the Sodium Perchlorate powder and add to a 1000 mL volumetric flask and fill to volume with reagent water. This will give you a stock standard at a concentration of 100 ppm (mg/L). Pipette 250 µL of this stock solution into a 250 mL volumetric flask and fill with reagent water to volume to get a concentration of 100 ppb

- IPCS – (See section 7.1.9 for added assistance) – Follow:

  [1] Prepare a laboratory fortified reagent blank containing Perchlorate at a concentration of 25 ppb by adding 12.5 µL of the 100 ppm stock solution to a 50 mL volumetric flask and filling to volume with reagent water.

  [2] Dissolve the following salts in reagent water to a final volume of 25.0 mL:

  - 1.0 g sodium chloride = 0.61 g Cl^-
  - 0.93 g sodium sulfate =0.63 g $SO_4^=$
  - 1.1 g sodium carbonate = 0.62 g $CO_3^=$

  [3] Remove 400 µL of the perchlorate in [1] and substitute it with 400 µL of the anion mixture in [2], This will give you a 25 ppb solution of perchlorate at the MCT (See Appendix II, Table III). Discard perchlorate waste in the assigned waste container.

- LRB – The reagent water from the MiliQ System.

- ICCS – Utilize the 4 ppb standard that was made for the generation of the calibration curve.

- LFB – Add 25 µL of 100 ppm stock perchlorate standard into a 100 mL volumetric flask and fill to volume with reagent water to make a concentration of 25 ppb.

- LFM – Add 2.5 mL of 100 ppm stock perchlorate solution to a 250 mL volumetric flask. Fill to volume to give an intermediary standard concentration of 1 ppm. Add 250 µL of

the 1 ppm intermediary standard to 5 mL of sample. This will render a matrix spike concentration of 50 ppb.

- LFMD – Repeat the same procedure as in the LFM, using another aliquot of the same sample matrix.
- CCCS – Utilize the mid point standard from the calibration curve.
- ECCS – Utilize the end point standard from the calibration curve.

7.2.3 Determine the conductivity of the field samples by using a calibrated conductivity meter. If the conductance of the sample is above the laboratory MCT, dilute/pretreat the sample before analysis or follow sponsor specifications regarding dilutions/pretreatments of project samples. Record the conductivity of all samples in Form No. 296, Sample Preparation Log.

7.2.3.1 Estimate the dilution factor by dividing the matrix conductance by the MCT. Round up this value to the next whole number and dilute the sample by a proportion equivalent to this value.

7.2.3.2 Measure the conductance of the diluted sample to confirm it to be below the MCT. Analyze the sample with the understanding that MRL has been elevated by a proportion equal to the dilution factor.

7.2.3.3 Pretreat the sample if the sponsor's objectives require monitoring below the concentration of the newly elevated MRL.

7.2.3.3.1 Carry out matrix pretreatment by employing three cartridges attached in series in the following order: Barium, Silver, and Hydrogen.

7.2.3.3.1.1 Rinse each cartridge individually with reagent water as per manufacturer's instructions.

7.2.3.3.1.2 Pretreat quality control samples first, i.e. the LRB and the LFB. Analyzing these samples will verify that the cartridges contribute no background interference. Rinse the cartridges further with a quadruple volume of reagent water if a response of more than the MDL is observed in the LRB.

7.2.3.3.1.3 Filter the concentrated sample through the series of cartridges at a flow rate of 1.0 mL/minute or less (approximately one drop/3-4 seconds). Follow the above flow rate carefully.

7.2.3.3.1.4 Discard the initial 3 mL fraction and then begin collecting the sample aliquot for analysis.

7.2.3.3.1.5 Prepare a LFM using pretreated sample in order to ensure data quality. Analyze the pretreated sample first and determine the Perchlorate concentration. Fortify another aliquot of the same sample with the Perchlorate concentrations close to, but greater than, the level determined in the native sample prior to the pretreatment. This step will rule out the presence of any effects caused by the matrix or confirm that the pretreatment was accurate. If the Perchlorate recovery falls outside the 80-120 %, then that particular sample should be reported as suspect matrix.

7.2.3.3.1.6 Measure the conductance of the pretreated sample and load the sample into an auto-sampler vial if the conductance is below the MCT. If the conductance is still higher than the MCT, repeat the pretreatment at a slower flow rate and by utilizing new cartridges.

7.2.4 Prepare the auto sampler vials according to section 7.1.2.3 of this SOP.

7.2.5 Load and analyze the standards and samples in the sample sequence provided in Table IV, Appendix II. Use this table to analyze one data batch. Additional batches may be added sequentially provided all QC requirements are met for each batch.

7.2.6 The IPC must be analyzed at the beginning of every analytical batch in conformance to section 9.3.2.1 of EPA Method 314.0. Additionally, the IPC must be:

1. measured for conductance and the measured conductance should not

exceed more than 10 % of the originally measured value. This value must be recorded in the Sample Preparation Log No. 296. If the measured conductance is found to be more than 10 %, a new IPC solution must be prepared.

2. the percent difference between the area/height of the IPC and the area/height of the LFB in the original IDC or the IPC in the previous analytical batch must be calculated using equation in section 7.1.9.8 of this SOP. The percent difference must not be greater than 25 %. If the percent difference is greater than 25 %, corrective action(s) must be taken according to section 9.3.2.5 of EPA Method 314.0.

3. the recovery of perchlorate must fall between 80 % and 120 %. If recovery is outside this criteria then corrective action must be taken according to section 9.3.2.5 of EPA Method 314.0.

4. the retention time of the IPC must be recorded in the Daily Run Log No.267 and care must be taken that the retention time does not shift more than 5 %. The column must be cleaned or replaced if the retention time shifts to less than 80 % of the originally recorded time (See section 9.3.2.4 of EPA Method 314.0).

8.0 QUALITY CONTROL CHECKS/ACCEPTANCE CRITERIA

Refer to Table V, Appendix III for a brief summary of the quality control acceptance criteria and Table VI, Appendix III for a summary of Perchlorate concentrations to be used.

8.1 Sample holding time must not exceeded 28 days

8.2 At least a five point calibration curve must be generated with the MRL set no lower than the lowest calibration standard. The instrument must be re-calibrated as soon as or as often as any of the acceptance criteria given below are not met.

8.3 An IPC must accompany every analysis batch. Conductance of IPC solution must be within ± 10% of original conductance value for MCT. The calculated percent difference between the IPC and the LFB in the original IDC /or the IPC in the previous analytical batch must be no greater than 25 %.The quantitated Perchlorate value must be between 80-120% of the fortified level (20 ppb to 30 ppb for a 25ppb fortification). The retention time of the IPC must not shift by more than 5 %.

8.4 Initial Calibration Check Standard (ICCS) must verify each calibration. Recovery must be 10% of the true value in order to continue with the analysis.

8.5 A Laboratory Fortified Blank (LFB) at 25 ppb must follow the ICCS. Recovery

must be 85-115 %. Sample batches that fail the LFB recovery limits must not be reported as valid.

8.6 A Continuing Calibration Check Standard (CCCS) and an End Calibration Check Standard (ECCS) at the mid and high level (alternating the two/10 samples) must continually monitor calibration. Recovery must be between 90-110%.

8.7 A Laboratory Reagent Blank (LRB), which must be analyzed prior to any sample, must be included in every analysis batch (up to 20 samples). The level of recovered perchlorate must be below the MDL in order to be accepted as a reagent blank. If it is >MDL, the analysis batch will not be considered valid.

8.8 A field or Laboratory Fortified Matrix (LFM), fortified matrix must accompany every sample batch. The LFM must be prepared at concentrations equal to or greater than the native sample concentration. The LFM should not be prepared at a concentration greater than 10 times the highest concentration observed in any field sample. If the recovery for Perchlorate is not between 80-120 %, and the recoveries for all other QC performance criteria are met, then the result for that LFM must be labeled as suspect matrix. Analyze also a duplicate of the LFM and calculate the Relative Percent Difference (RPD). RPD must be ± 15%. If the control criteria are not met for matrix spike results, all samples in the analytical batch must be re-analyzed. If the LFM fails the recovery criteria even after re-analysis, label sample as suspect matrix. If the control criteria are not met for the second matrix spike analysis, the results associated with the best matrix spike analysis shall be qualified “N” and reported.

8.9 For samples that are pretreated the LRB and the LFB must also be pretreated. A special LFM must be prepared and pretreated. All three samples must adhere to the same recovery criteria as the untreated LRB, LFB, and the LFM.

9.0 WASTE DISPOSAL

The Perchlorate waste must be discarded in the containers provided by the Texas Tech Safety Services. The waste will be disposed according to the Texas Tech University Environmental Health And Safety guidelines.

10.0 LITERATURE CITED

10.1 Test Methods for Evaluating Solid Wastes, SW-846, Third Edition, through update II, September 1994.

10.2 “Determination of Perchlorate In Drinking Water by Ion Chromatography.” U.S. EPA Method 314.0., Revision 1.0 November 1999, National Exposure Research Laboratory Office of Research and Development, Cincinnati, Ohio.

10.3 “Determination of Perchlorate Using Ion Chromatography with Chemical Suppression Conductivity Detection.” U.S. EPA Method 9058, Revision 0,

November 2000.

11.0 APPENDICES

Appendix I: Table I, Ion Chromatograph Operating Procedures.

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12.0 DEFINITIONS

MDL – Method Detection Limit

MCT – Matrix Conductivity Threshold

QCS – Quality Control Sample

IPCS – Instrument Performance Check Standard

LRB – Laboratory Reagent Blank

ICCS – Initial Calibration Check Standard

LFB – Laboratory Fortified blank

LFM – Laboratory Fortified Matrix

LFMD – Laboratory Fortified Matrix Duplicate

CCCS – Continuing Calibration Check Standard

ECCS – End Calibration Check Standard

APPENDIX I

Table I: Ion Chromatograph Operating Parameters

Ion Chromatograph:	Dionex IC25
Guard Column:	IonPac AG16 (4 x 50mm)
Analytical column:	IonPac AS16 (4 x 250)
Eluent:	100 mM NaOH
Eluent Flow:	1.0 mL /minute
Injection Volume:	1000 µL
Typical System Backpressure:	1200 – 2000 psi
Detection:	Suppressed conductivity, external water mode
Background Conductivity:	< 2-5 µS
Determined MCT:	3318 µS
Total method analysis time:	11 – 14 minutes

APPENDIX II

Table II: Preparation of Calibration Standards

Volume of stock Perchlorate standard, at 100ppm, (microliters) used	Volumetric Flask (mL)	Final concentration (µg/L or ppb)
10	250	4
125	250	50
250	250	100
375	250	150
500	250	200

Table III: Preparation of common anion mixture with Perchlorate

Volume of common anion mixture (milliliters)	Perchlorate stock, at 100ppm, added (microliters-µL)	Final concentration of Perchlorate in 50 mL volume
0 µL	12.5	25.0 µg/L (ppb)
0.20 = 200µL	12.5	25.0 µg/L (ppb)
0.30 = 300µL	12.5	25.0 µg/L (ppb)
0.40 = 400µL	12.5	25.0 µg/L (ppb)
0.50 = 500µL	12.5	25.0 µg/L (ppb)
0.60 = 600µL	12.5	25.0 µg/L (ppb)
0.80 = 800µL	12.5	25.0 µg/L (ppb)
1.00 = 1000µL	12.5	25.0 µg/L (ppb)

Table IV: Sample Analysis Sequence Table

Injection #	Sample Description	Acceptance Criteria
1	(IPCS) Instrument Performance Check Standard at MCT (25 ppb at 3318 µS/cm)	Recovery of 80-120%
2	QCS at calibration midpoint (100ppb)	Recovery of 90-110%
3	(LRB)Laboratory Reagent Blank	< MDL check sample
4	ICCS at the MRL (4.0 ppb)	Recovery of 90-110%
5	(LFB)Laboratory Fortified Blank (25ppb)	Recovery of 85-115%
6	Sample 1	Normal analysis
7	Sample 2	Normal analysis
8	Sample 3	Normal analysis
9	Sample 4	Normal analysis
10	Sample 5	Normal analysis
11	Sample 6	Normal analysis
12	Sample 7	Normal analysis
13	Sample 8	Normal analysis
14	Sample 9	Normal analysis
15	Sample 10	Normal analysis
16	CCCS (100ppb)	Recovery of 90-110
17	Sample 11	Normal analysis
18	Sample 12	Normal analysis
19	Sample 13	Normal analysis
20	Sample 14	Normal analysis
21	Sample 15	Normal analysis
22	Sample 15	Normal analysis
23	Sample 16	Normal analysis
24	Sample 17	Normal analysis
25	Sample 18	Normal analysis
26	Sample 19	Normal analysis
27	Sample 20	Normal analysis
28	Sample 20 - LFM(50ppb)	Recovery of 90-110%
29	Sample 20 - LFMD (50ppb)	RPD ± 15%
30	ECCS (200ppb)	Recovery of 90-110

APPENDIX III

Table V: Summary of Quality Control Acceptance Criteria

Required Analysis	Required Percent Recovery Limits
QCS	90-110%
IPCS	80-120%
MCT	80-120%
ICCS	90-110%
LFB	85-115%
LFM	80-120%
CCC	90-110%
ECCS	90-110%

Table VI: Summary of Preparations of Perchlorate Fortifications

Required Analysis	Perchlorate Concentration
MDL	2.5 ppb
QCS	100 ppb
IPC	25 ppb
MCT	25 ppb
ICCS	4.0 ppb
LFB	25 ppb
LFM	50 ppb
CCCS	100 ppb and 200 ppb
ECCS	100 ppb and 200 ppb

APPENDIX IV

Table VII: Determination of Method Detection Limit And Retention Time Window

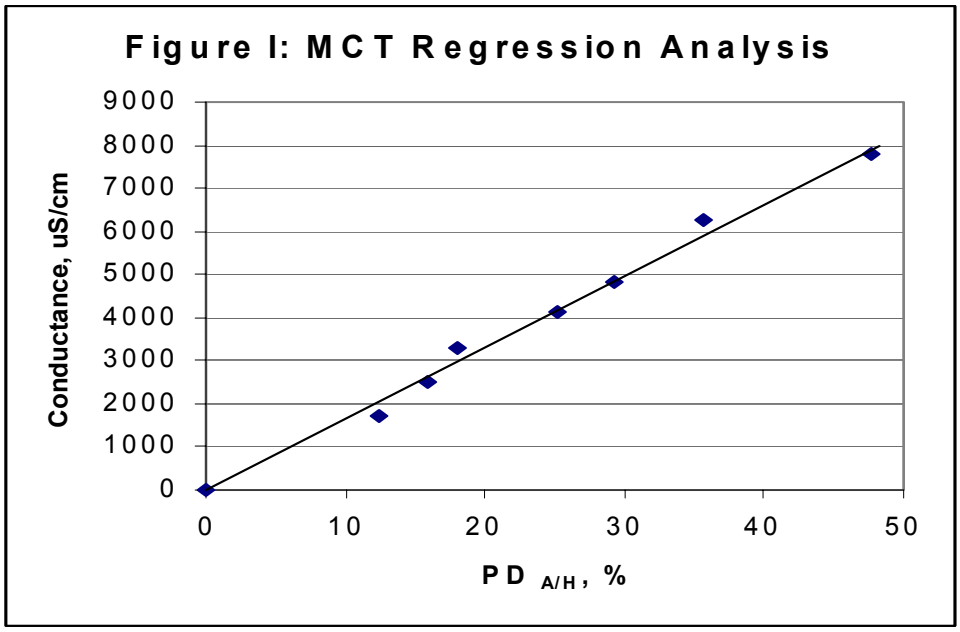
Perchlorate Actual Conc. (µg/L)	Retention Time (mins)	Perchlorate Measured Conc. (µg/L)	Std Deviation (S_{n-1})	MDL
2.5	8.96	2.02	0.164	0.5
2.5	8.67	2.09		
2.5	8.95	2.35		
2.5	8.92	2.13		
2.5	8.69	1.95		
2.5	8.69	1.91		
2.5	8.92	2.28		
	Average = 8.82			

Table VIII: Calculation of MDL

Sample Number, n	**Measured Value, mg/L**	$\mathbf{X_i - \bar{X}}$	$\mathbf{(X_i - \bar{X})^2}$
1	2.02	-0.08	0.006
2	2.09	-0.01	0.000
3	2.35	0.25	0.063
4	2.13	0.03	0.001
5	1.95	-0.15	0.023
6	1.91	-0.19	0.036
7	2.28	0.18	0.032
∑	14.7	---	0.161
X	2.10	Blank < ½ MDL	
s	0.164	S = √ 0.161/6	
MDL	mg/L	MDL = 0.5	

Table IX: Determination of Maximum Conductivity Threshold

Sample	Conductivity µS/cm	RT min	Measured ClO_4^-, µg/L	% Rec	Area	Height	A/H ratio	$PD_{A/H}$ %
Perchlorate fortified at 25 µg/mL								
LFB	<1	8.87	23.4	94	0.0673	0.364	0.185	0.00
MA (200)	1736	8.78	23.1	92	0.0665	0.320	0.208	12.4
MA (300)	2508	8.78	23.8	95	0.0687	0.321	0.214	15.8
MA (400)	3309	8.74	23.2	93	0.0670	0.307	0.218	18.0
MA (500)	4108	8.75	22.6	90	0.0650	0.281	0.231	25.1
MA (600)	4835	8.73	22.3	89	0.0643	0.269	0.239	29.3
MA (800)	6269	8.71	21.6	86	0.0622	0.248	0.251	35.7
MA (1000)	7805	8.68	20.9	84	0.0603	0.221	0.273	47.6



$Y = 168.95\ X - 60.69$, $R^2 = 0.9923$ where, Y=MCT

Therefore, MCT = [(168.95 x 20)-60.69] = 3,318µS/cm

SOP Reauthorization			
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Reviewer Signature(s)	Date	Director Signature	Date

The Institute of Environmental and Human Health (TIEHH)
Texas Tech University
Texas Tech University Health Sciences Center
Box 41163, Lubbock, TX 79409-1163
qa@tiehh.ttu.edu

Extraction and Cleanup of Tissue Samples to be Analyzed for Perchlorate Using Ion Chromatography

Author(s):	_____ Todd Anderson	_____ Date
QA Review:	_____ Heather Winn	_____ Date
Asst. Director for Science:	_____ Lou Chiodo	_____ Date
Director:	_____ Ronald J. Kendall	_____ Date

1.0 OBJECTIVE

To describe the procedures for (1) extracting tissue samples and (2) cleaning up extracts of tissue samples to be analyzed for the perchlorate anion. Samples will be extracted using the accelerated solvent extraction (ASE) and analyzed for perchlorate using ion chromatography (IC).

2.0 HEALTH AND SAFETY

Proper lab attire including scrubs, lab coat, gloves, and safety glasses should be worn at all times.

3.0 PERSONNEL/TRAINING RESPONSIBILITIES

Any TIEHH employee familiar with the equipment and laboratory techniques and trained in this and referenced SOPs may perform this procedure. Users of this method must demonstrate the ability to generate acceptable results, using the procedures described herein.

4.0 REQUIRED AND RECOMMENDED MATERIALS

4.1 Materials

stainless steel extraction cell
Collection vial (60 mL)
Cellulose filter
Glass beakers (50 mL)
Oven
Scissors
Graduated cylinder (50 mL)
SOP AC-4-04-01

Volume adjustable pipetter — Disposable pipet tips
SPE columns — SPE vacuum manifold
Analytical balance — Polypropylene funnel
Filters (Gelman IC Acrodisc, P/N 4485, or equivalent)

4.2 Reagents

milli-Q water (18 mΩ)

5.0 PROCEDURES

5.1 Summary of Method

A measured weight of air-dried tissue sample is extracted using water as the solvent and accelerated solvent extraction (ASE). A portion of the extract (typically ≤ 1 mL) is cleaned using solid phase extraction (SPE) to remove interferences in the extract. The choice of SPE varies with the type of extract as certain phases provide better cleanup than others for certain sample types (e.g. As reported in the literature (Ellington and Evans, 2000), alumina works well with plant extracts). Analysis is completed by injecting a dilution of the cleaned extract into an ion chromatograph equipped with a conductivity detector.

Before operating the ASE, users should be familiar with SOP AC-4-04-01.

All glassware and materials should be rinsed with milli-Q (18 mΩ) water. Users of this SOP should refer to SOP AC-1-01-01.

5.2 Solvent Selection and Preparation for ASE.

5.2.1 Fill the solvent reservoir on the ASE with milli-Q (18 mΩ) water.
5.2.2 Make sure the in-line filter in the solvent reservoir rests on the bottom of the reservoir to prevent air from being drawn through the line.
5.2.3 Hand-tighten the lock ring cap on the solvent reservoir.

5.3 Sample Preparation

5.3.1 Weigh out 1- 5 grams of sample. Record the wet weight of the sample.
5.3.2 Allow sample to air dry (overnight), then record dry weight.
5.3.3 Thoroughly mince the air-dried sample with scissors.
5.3.4 Prepare extraction cells by inserting a cellulose filter into the cell.
5.3.5 Load sample into cell body with funnel. Be careful to keep cell body threads clean.
5.3.6 Hand-tighten top cap of cell body.

5.4 Extraction

5.4.1 Place cell or cells in the ASE cell tray with the “Dionex” symbol on top.
5.4.2 Place collection vials in the ASE vial tray.
5.4.3 Use the following conditions for extraction:

Preheat = 0 min. Pressure = 1500 psi
Static = 1min. Temperature = 100 C°

Flush = 50% Water = 100%
Purge = 60 sec. Cycle =1

5.4.4 Load the method or schedule created.
5.4.5 Measure and record the volume of extract obtained from each extraction.

5.5 Sample Cleanup

5.5.1 Select the appropriate SPE cartridge for the sample.

laboratory fish: silica (conditioned with milli-Q water)
vegetation: alumina (conditioned with milli-Q water)
tadpoles: silica (conditioned with milli-Q water)
mammal (liver, kidney, thyroid): silica (conditioned with milli-Q water)
blood: precipitation with ethanol, Dionex OnGuard-Ba, Ag, and H
lab crayfish: C18, powdered alumina
damselflies: silica (conditioned with milli-Q water)
field fish: alumina (conditioned with milli-Q water)
birds: alumina (conditioned with milli-Q water)

5.5.2 Condition the SPE cartridge as appropriate.
5.5.3 Place a labeled collection vial in the appropriate slot within the vacuum manifold for collection.
5.5.4 Replace top of manifold, making sure needle is in the collection vial.
5.5.5 Pipette 0.5 - 1 mL of sample extract into the SPE column.
5.5.6 Slowly turn on vacuum and allow sample to completely elute into the collection vial.
5.5.7 Completely elute 4 - 4.5 mL of milli-Q water through the SPE column.
5.5.8 Turn off vacuum, remove top of manifold, and remove collection vial.
5.5.9 Filter the eluate using an IC filter (0.45 μm).

6.0 QUALITY CONTROL CHECKS/ACCEPTANCE CRITERIA

Sample blanks (sand) and matrix spikes should be incorporated into the extraction procedure. The use of perchlorate-spiked extracts is also recommended to evaluate potential loss of perchlorate during cleanup.

7.0 LITERATURE

Anderson, T. A., and T. H. Wu. 2002. Extraction, cleanup, and analysis of the perchlorate anion in tissue samples. Bulletin of Environmental Contamination and Toxicology. 68:684-691.

Ellington JJ, Evans JJ (2000) Determination of perchlorate at parts-per-billion levels in plants by ion chromatography. J Chrom A 898:193-199

SOP Reauthorization

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